

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
11 January 2001 (11.01.2001)

PCT

(10) International Publication Number  
**WO 01/02021 A2**

- (51) International Patent Classification<sup>7</sup>: A61K 51/00
- (21) International Application Number: PCT/IB00/01260
- (22) International Filing Date: 5 July 2000 (05.07.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
P 9901489 5 July 1999 (05.07.1999) ES
- (71) Applicant and  
(72) Inventor: ORTIZ ARMUA, Pedro [ES/ES]; Dr. Severo Ochoa, 37, Local 4-I, E-28100 Alcobendas (ES).
- (74) Agent: TORNER LASALLE, Núria; Alberes, 2, E-08017 Barcelona (ES).
- (81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

## Published:

- Without international search report and to be republished upon receipt of that report.

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



A2

WO 01/02021

(54) Title: A RADIOLABELED MAMMALIAN TACHYKININ PEPTIDE ANALOGUE

(57) Abstract: A radiolabeled mammalian tachykinin peptide analogue; use of the analogue for mammalian *in vivo* tachykinin peptide receptor imaging; and a diagnostic kit comprising the analogue.

WO 01/02021

PCT/IB00/01260

1

## TITLE

A radiolabeled mammalian tachykinin peptide analogueField of Invention:

5 The present invention relates to a radiolabeled mammalian tachykinin peptide analogue; use of the analogue for mammalian *in vivo* tachykinin peptide receptor imaging; and a diagnostic kit comprising the analogue.

Description of the Background Art:

10 Tachykinins are a family of peptides that share a common C-terminal amino acid sequence, -Phe-X-Gly-Leu-Met-NH<sub>2</sub>, where X represents either Phe, Ile, or Val.

The mammalian tachykinins include substance P (SP), neurokinins A and B and two N-terminally extended forms of neurokinin A, i.e. neuropeptide K and neuropeptide Y. Currently accepted tachykinin receptor nomenclature defines three homologous receptor types: (a) 15 the neurokinin 1 receptor, preferring substance P, (b) the neurokinin 2 receptor, preferring neurokinin A, and (c) the neurokinin 3 receptor, preferring neurokinin B.

The tachykinin receptors have a wide tissue distribution, and interaction with their ligands is associated with diverse responses, such as immunological responses, histamine release, inflammation, nerve regeneration and wound healing (P.M. van Hagen, "Somatostatin and Substance P analogues: Applications on autoimmune and haematological diseases", Thesis Erasmus, University of Rotterdam (1995), Chapter 8.1; Breeman et al. J. of Nuclear Medicine 20:37:108-117 (1996)).

Receptor imaging *in vivo* using a peptide labeled with a radioisotopes is known in the art.

25 Breeman et al. J. of Nuclear Medicine 37:108-117 (1996); and Hagen et al. European J. of Medicine 23:1508-1513 (1996) describe a <sup>111</sup>In labeled SP peptide and the use of this for SP receptor imaging.

Hennig et al. Int. J. Cancer 61:786-792 (1992); and Walsh et al. Annals of the Rheumatic Diseases 51:313-317 (1992) describe a <sup>125</sup>I labeled SP peptide and the use of this for SP receptor imaging.

30 Hagen et al. states in the abstract "Degradation of <sup>111</sup>In..-SP started in the first minutes after administration, resulting in a half-life of 10 min..".

WO 01/02021

PCT/IB00/01260

2

Below it is said "We conclude that, despite its short half-life,  $^{111}\text{In}$ ..-SP,..., can be used to visualize the thymus.

**Summary of invention:**

5 The problem to be solved by the present invention is to provide a radiolabeled mammalian tachykinin peptide analogue which has a relatively long half-life *in vivo*.

The solution is a radiolabeled mammalian tachykinin peptide analogue comprising a tachykinin peptide labeled with a  $^{99\text{m}}\text{Tc}$  isotope ( $^{99\text{m}}\text{Tc}$ -tachykinin peptide) and which has a half-life *in vivo* of at least 30 minutes, preferably a half-life *in vivo* is of at least 1 hour, more 10 preferably a half-life *in vivo* is of at least 3 hours, and most preferably a half-life *in vivo* is of at least 5 hours.

One advantage of a tachykinin peptide analogue as described herein is that the relative long half-life will facilitate its use in e.g. *in vivo* receptor imaging.

15 A further advantage is that it does forms less aggregates as compared to prior art known radiolabeled tachykinin analogues.

As stated above it is known in the art to perform receptor imaging *in vivo* using a tachykinin peptide labeled with a  $^{111}\text{In}$  or a  $^{125}\text{I}$  radioisotope. (Breeman et al. J. of Nuclear Medicine 37:108-117 (1996); Hagen et al. European J. of Medicine 23:1508-1513 (1996); Henning et al. Int. J. Cancer 61:786-792 (1992); and Walsh et al. Annals of the Rheumatic Diseases 20 51:313-317 (1992)).

A radiolabeled tachykinin analogue as described herein can be used in a similar way as e.g. described in above mentioned references. Further, the improved half-life will facilitate this use in relation to receptor imaging.

Accordingly, the present invention relates in a second aspect to use of a radiolabeled 25 tachykinin analogue as described herein for mammalian *in vivo* tachykinin peptide receptor imaging.

In a third aspect, the present invention relates to a diagnostic kit comprising a radiolabeled tachykinin analogue as described herein and which is suitable to be used in any of the uses of the radiolabeled tachykinin analogue as described herein.

30 The preparation of such a diagnostic kit having special suitable extra ingredients is within the skilled persons general knowledge.

WO 01/02021

PCT/IB00/01260

**Definitions:**

Prior to a discussion of the detailed embodiments of the invention is provided a definition of specific terms related to the main aspects of the invention.

The term "tachykinin peptide" are a common known term in the art for a family of peptides. See above and e.g. Breeman et al. (J. of Nuclear Medicine 37:108-117 (1996)). These peptides are well defined also at amino acid levels (see below) and characterized by their specific binding to important mammalian receptors (see above). The term "tachykinin peptide" denotes herein these well known peptides in their natural form, i.e. with their natural amino acid sequences. However, it is well known in the art that a polypeptide sequence may be modified in the sense of a change in the amino acid sequence without a significant change in its essential characteristics. An essential characteristic of a tachykinin peptide as described herein is its binding affinity to the receptor which has the specific tachykinin peptide as preferred substrate/ligand. As stated above (a) the neurokinin 1 receptor has substance P as preferred substrate, (b) the neurokinin 2 receptor has neurokinin A as preferred substrate, and (c) the neurokinin 3 receptor has neurokinin B as preferred substrate. Accordingly, beside denoting these well known tachykinin peptides in their natural form, the scope of the term "tachykinin peptide" also includes an amino acid changed variant of these peptides which has maintained essentially the same binding affinity for the receptor. As illustration, the tachykinin peptide substance P (SP) is the preferred substrate for the receptor neurokinin 1. SP has a binding affinity of  $K_d = 0.5\text{-}1.0 \text{ nM}$  for this receptor (see P.M. van Hagen, "Somatostatin and Substance P analogues: Applications on autoimmune and haematological diseases", Thesis Erasmus, University of Rotterdam (1995), chapter 7; section "Substance P"). Consequently, a SP variant which has maintained essentially the same binding affinity for the receptor neurokinin 1 is within the scope of the term "tachykinin peptide" as described herein.

The term "<sup>99m</sup>Tc" denotes the <sup>99m</sup>Technetium isotope (Molinski V.J. Int. J. Appl. Radiat. Isot. 33:811-819 (1982)). The nomenclature "<sup>99m</sup>Tc" and "Tc-99m" may be used herein interchangeably.

The measurement of the term "half-life" is determined according to standard assays in a human. Preferably, the assay determines a time course of total (<sup>99m</sup>Tc- + <sup>99m</sup>Tc-tachykinin peptide) and peptide bound (<sup>99m</sup>Tc-tachykinin peptide) radioactivity in human blood plasma at selected time periods up to 50 hours. Based on this the half-life is calculated. It is within the skilled persons general knowledge to identify a suitable strategy for separating the non peptide

WO 01/02021

PCT/IB00/01260

4

bound radioisotope “<sup>99m</sup>Tc-“ and the peptide bound radioisotope “<sup>99m</sup>Tc-tachykinin peptide” in the blood plasma. An example of a suitable strategy is described below.

Radioactivity in blood plasma is measured with an LKB-1282-Compugamma system or a Geli-detector equipped with a multichannel analyser (Series 40, Canberra). Blood is collected in EDTA-containing tubes immediately cooled on ice and the samples are immediately centrifuged at 0°C, and plasma is fractionated on SEP-PAK C<sub>18</sub> columns. Using the separation technique described in (Breeman et al. Eur. J. Nucl. Med. 21:328-335 (1994)), <sup>99m</sup>Tc-tachykinin peptide binds to SEP-PAK C<sub>18</sub> stationary phase and is only eluted with ethanol, while free <sup>99m</sup>Tc is not retained on SEP-PAK C<sub>18</sub> columns. The radioactivity in plasma, which is eluted with ethanol from the SEP-PAK C<sub>18</sub> columns, is termed peptide bound radioactivity. Total amount of radioactivity is the sum of peptide bound radioactivity and free <sup>99m</sup>Tc related radioactivity. Blood samples are collected directly 2, 5, 10, 20, and 40 min, 1, 4, 10, 20, and 50 hours after infusion.

15 Embodiment(s) of the present invention is described below, by way of example(s) only.

**Detailed description of the invention:**

**A radiolabeled tachykinin analogue:**

20 An embodiment of the invention relates to a tachykinin peptide analogue, wherein the tachykinin peptide is selected from the group consisting of: a neurokinin A peptide; a neurokinin B peptide; a neuropeptide K (a N terminally extended form of neurokinin A); a neuropeptide Y (a N terminally extended form of neurokinin A); and preferably a substance P (SP) peptide.

25 Example 2 herein (*vide infra*) shows that a <sup>99m</sup>Tc-SP peptide analogue is capable of performing a specific binding in the salivary glands of a mice of 0.62% injected dose per gram organ (%ID/g).

Accordingly, an embodiment relates to a tachykinin peptide analogue, as described herein, wherein the analogue is capable of performing a specific binding in the salivary glands 30 of a mice of at least 0.35% injected dose per gram organ (%ID/g), preferably wherein the analogue is capable of performing a specific binding in the salivary glands of a mice of at least 0.45% injected dose per gram organ (%ID/g); more preferably wherein the analogue is capable

WO 01/02021

PCT/IB00/01260

5

of performing a specific binding in the salivary glands of a mice of at least 0.55% injected dose per gram organ (%ID/g), expressed as the difference in tissue uptake (90 minutes uptake) between untreated mice and mice treated with 90 nmol of a non-radioactive tachykinin peptide.

In relation to this specific binding in the salivary glands the tachykinin peptide is preferably a substance P (SP) peptide.

As described above a tachykinin peptide may be described by a common C-terminal sequence.

Accordingly, an embodiment of the invention relates to a tachykinin peptide analogue as described herein, wherein the tachykinin peptide comprises the C-terminal amino acid sequence, -Phe-X-Gly-Leu-Met-NH<sub>2</sub>, where X represents either Phe, Ile, or Val (SEQ ID NO 1).

The natural form of the substance P (SP) peptide has the amino acid sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub> (Breeman et al. J. of Nuclear Medicine 37:108-117 (1996)).

An embodiment therefore relates to a tachykinin peptide analogue as described herein, wherein the tachykinin peptide is a substance P (SP) peptide consisting essential of the amino acid sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub> (SEQ ID NO 2).

A preferred way of making a radiolabeled tachykinin analogue as described herein is wherein the <sup>99m</sup>Tc isotope is labeled to the tachykinin peptide through a linking molecule situated between the tachykinin peptide and the <sup>99m</sup>Tc isotope.

Accordingly, an embodiment relates to a radiolabeled tachykinin analogue as described herein, wherein the <sup>99m</sup>Tc isotope is labeled to the tachykinin peptide through a linking molecule situated between the tachykinin peptide and the <sup>99m</sup>Tc isotope.

Examples of suitable linking molecules are a 1-imino-4-mercaptopbutyl molecule; a DTPA molecule; or a 3-(p-Hydroxyphenyl)propinyl molecule.

The use DTPA and 3-(p-Hydroxyphenyl)propinyl as a linking molecule is described by Breeman et al. (J. of Nuclear Medicine 37:108-117 (1996)). A radiolabeled tachykinin analogue as described herein, may be made by use of one of these molecules as linking molecules and using the strategy of the "Materials and Method" section of Breeman et al., combined with the skilled persons knowledge, wherein the radioisotope <sup>111</sup>In of Breeman et al. is changed to the radioisotope <sup>99m</sup>Tc.

Working example 1 herein describes a method for making a <sup>99m</sup>Tc-SP peptide analogue using a 1-imino-4-mercaptopbutyl molecule as linking molecule.

WO 01/02021

PCT/IB00/01260

6

Accordingly, an aspect of the invention relates to a method for making a radiolabeled  $^{99m}\text{Tc}$ -SP peptide comprising:

- (a) linking a 1-imino-4-mercaptopropyl to a SP peptide;
- (b) removing excess linker molecule by e.g. gel chromatography; and
- 5 (c) labeling with  $^{99m}\text{Tc}$ .

Use of a radiolabeled tachykinin:

As described above a radiolabeled tachykinin analogue as described herein may be used for mammalian *in vivo* tachykinin peptide receptor imaging.

10 Preferably, the use is wherein the tachykinin receptor is a neurokinin 1 receptor expressed by arterioles and/or venules located in the submucosa, muscularis mucosa, external longitudinal muscle, and/or serosa, and in relation to this it is preferred that the tachykinin peptide is a substance P (SP) peptide since SP is the preferred substrate for the neurokinin 1 receptor. See above and (P.M. van Hagen, "Somatostatin and Substance P analogues: Applications 15 on autoimmune and haematological diseases", Thesis Erasmus, University of Rotterdam (1995), Chapter 8.1, "Introduction").

20 Further embodiments relate to the use as described herein, wherein the tachykinin receptor is a neurokinin 2 receptor and wherein the tachykinin peptide is a neurokinin A peptide; or wherein the tachykinin receptor is a neurokinin 3 receptor and wherein the tachykinin peptide is a neurokinin B peptide.

An embodiment of the invention relates to the use as described herein, wherein the *in vivo* tachykinin receptor imaging is done *in vivo* in a human.

25 Hagen et al. European J. of Medicine 23:1508-1513 (1996); Hennig et al. Int. J. Cancer 61:786-792 (1995); Walsh et al. Annals of the Rheumatic Diseases 51:313-317 (1992); Ruff et al. Peptides 6:107-111 (1985) describe such receptor imaging in a human using  $^{111}\text{In}$  or  $^{125}\text{I}$  labeled SP peptide analogues.

Among others, based on the teaching of these references it is within the skilled persons general knowledge to perform *in vivo* tachykinin receptor imaging *in vivo* in a human by use of a  $^{99m}\text{Tc}$  labeled tachykinin peptide analogue as described herein.

30 An further embodiment relates to the use as described herein, wherein the human *in vivo* tachykinin receptor imaging is done in order to measure a specific amount of a tachykinin receptor situated *in vivo* on a human cell.

WO 01/02021

PCT/IB00/01260

7

To measure specific amounts of the receptor may be specially useful for diagnostic purposes, since specific changes in the amount of receptor may correspond to a specific disease.

In relation to this it is preferred that the human cell is a human cell selected from the group consisting of a tumor cell, a T-lymphocyte cell, a thymus cell, a neoplasm cell, a monocyte cell, and a mast cell.

These cells are known to express in particular the neurokinin 1 receptor (Hagen et al. European J. of Medicine 23:1508-1513 (1996); Hennig et al. Int. J. Cancer 61:786-792 (1995); Walsh et al. Annals of the Rheumatic Diseases 51:313-317 (1992); Ruff et al. Peptides 6:107-111 (1985)).

Accordingly, in relation to these specific human cells it is preferred that the tachykinin receptor is a neurokinin 1 receptor and the tachykinin peptide is a substance P (SP) peptide.

Final embodiments relates to the use as described herein, wherein the purpose of the tachykinin receptor imaging is a diagnostic purpose, and in particular wherein the diagnostic purpose is a diagnostic relating to inflammation or a diagnostic relating to a tumor.

It is clear from the references cited above that a radiolabeled mammalian tachykinin peptide analogue is highly useful for such diagnostic purposes in particular due to it provides the possibility of measure a specific amount of a tachykinin receptor situated *in vivo* on a human cell.

20 **Examples:**

**Example 1:**

Preparation of a <sup>99m</sup>Tc isotope labeled SP peptide using 1-imino-4-mercaptopbutyl molecule as linking molecule:

All essential ingredients used in this example are commercially available.

A high specific activity Tc-99m-SP was prepared using a 1-imino-4-mercaptopbutyl group linked between reduced Tc-99m and SP. A formulation of 30 nmol SP, 1 µmol of 2-iminothiolane (2-IT) and 22 µmol of SnCl<sub>2</sub> resulted with a Tc-99m-SP labeling efficiency of >90% and stable over 6 hours. Prior to labeling with Tc-99m, the excess unreacted linker molecule was removed from modified SP by gel chromatography and the reaction vials were stored at -20°C. 64.7 ± 5.9% of Tc-99m was dissociated from labeled molecule following 1 hour incubation of Tc-99m labeled SP with 500:1 molar excess of cysteine.

WO 01/02021

PCT/IB00/01260

8

This 2-IT derivatization method is a simple, efficient and amenable to a formulation for the preparation of Tc-99m labeled SP analogues.

**Example 2:**5 Specific binding/uptake in the salivary glands of a mice of Tc-99m-SP:

Mice: a commercial available laboratory mice

90 minutes Biodistribution of Tc-99m-SP in mice as a percentage of injected dose per gram tissue (%ID/g). Values are mean (%ID/g) and standard error of the mean.

Blood	Spleen	Liver	Lung	Kidney
2.2±0.1	7.5±1.2	15.2±1.8	2.4±0.4	10.1±1.5

10

90 minutes uptake of Tc-99m-SP in salivary glands of mice as a percentage of injected dose per gram organ (%ID/g) before and after non-radioactive SP administration. Values are mean (%ID/g) and standard error of the mean.

Control	90 nmol SP	Specific uptake
1.60±0.05	0.98±0.05	0.62

WO 01/02021

PCT/IB00/01260

**CLAIMS**

1. A radiolabeled mammalian tachykinin peptide analogue comprising a tachykinin peptide labeled with a  $^{99m}\text{Tc}$  isotope ( $^{99m}\text{Tc}$ -tachykinin peptide) and which has a half-life *in vivo* of at least 30 minutes, preferably a half-life *in vivo* is of at least 1 hour, more preferably a half-life *in vivo* is of at least 3 hours, and most preferably a half-life *in vivo* is of at least 5 hours.
- 5 2. The tachykinin peptide analogue of claim 1, wherein the tachykinin peptide is selected from the group consisting of: a neurokinin A peptide; a neurokinin B peptide; a neuropeptide K (a N terminally extended form of neurokinin A); a neuropeptide Y (a N terminally extended form of neurokinin A); and preferably a substance P (SP) peptide.
- 10 3. The tachykinin analogue of claims 1 or 2, wherein the analogue is capable of performing a specific binding in the salivary glands of a mice of at least 0.35% injected dose per gram organ (%ID/g), expressed as the difference in tissue uptake (90 minutes uptake) between untreated mice and mice treated with 90 nmol of a non-radioactive tachykinin peptide.
- 15 4. The tachykinin analogue of claims 3, wherein the analogue is capable of performing a specific binding in the salivary glands of a mice of at least 0.45% injected dose per gram organ (%ID/g); more preferably wherein the analogue is capable of performing a specific binding in the salivary glands of a mice of at least 0.55% injected dose per gram organ (%ID/g).
- 20 5. The tachykinin analogue of claims 3 or 4, wherein the tachykinin peptide is a substance P (SP) peptide.
6. The tachykinin peptide analogue of any of claims 1-5, wherein the tachykinin peptide comprises the C-terminal amino acid sequence, -Phe-X-Gly-Leu-Met-NH<sub>2</sub>, where X represents either Phe, Ile, or Val (SEQ ID NO 1).
- 25 7. The tachykinin peptide analogue of any of claims 1-6, wherein the tachykinin peptide is a substance P (SP) peptide consisting essential of the amino acid sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH<sub>2</sub> (SEQ ID NO 2).
8. The radiolabeled tachykinin analogue of any of claims 1-7, wherein the  $^{99m}\text{Tc}$  isotope is labeled to the tachykinin peptide through a linking molecule situated between the tachykinin peptide and the  $^{99m}\text{Tc}$  isotope.
- 30 9. The radiolabeled tachykinin analogue of claim 8, wherein the linking molecule is a 1-imino-4-mercaptobutyl molecule.

WO 01/02021

PCT/IB00/01260

10

10. The radiolabeled tachykinin analogue of claim 8, wherein the linking molecule is a DTPA molecule.
11. The radiolabeled tachykinin analogue of claim 8, wherein the linking molecule is a 3-(p-Hydroxyphenyl)propinyl molecule.
- 5 12. Use of a radiolabeled tachykinin analogue of any of claims 1-11 for mammalian *in vivo* tachykinin peptide receptor imaging.
13. The use of claim 12, wherein the tachykinin receptor is a neurokinin 1 receptor expressed by arterioles and/or venules located in the submucosa, muscularis mucosa, external longitudinal muscle, and/or serosa.
- 10 14. The use of claim 13, wherein the tachykinin peptide is a substance P (SP) peptide.
15. The use of claim 12, wherein the tachykinin receptor is a neurokinin 2 receptor and wherein the tachykinin peptide is a neurokinin A peptide.
16. The use of claim 12, wherein the tachykinin receptor is a neurokinin 3 receptor and wherein the tachykinin peptide is a neurokinin B peptide.
- 15 17. The use of any of claims 12-16, wherein the *in vivo* tachykinin receptor imaging is done *in vivo* in a human.
18. The use of claim 17, wherein the human *in vivo* tachykinin receptor imaging is done in order to measure a specific amount of a tachykinin receptor situated *in vivo* on a human cell.
19. The use of claim 18, wherein the human cell is a human cell selected from the group consisting of a tumor cell, a T-lymphocyte cell, a thymus cell, a neoplasm cell, a monocyte cell, and a mast cell.
- 20 20. The use of claim 19, wherein tachykinin receptor is a neurokinin 1 receptor and wherein the tachykinin peptide is a substance P (SP) peptide.
21. The use of any of claims 12-20, wherein the purpose of the tachykinin receptor imaging is a diagnostic purpose.
- 25 22. The use of claim 21, wherein the diagnostic purpose is a diagnostic relating to inflammation or a diagnostic relating to a tumor.
23. A diagnostic kit comprising a radiolabeled tachykinin analogue of any of claims 1-11 and which is suitable to be used in any of the uses of claims 12-22.

WO 01/02021

PCT/IB00/01260

1

**Sequence listing:**

&lt;170&gt; PatentIn Ver. 2.1

5 <210> 1  
<211> 5  
<212> PRT  
<213> Human10 <220>  
<221> VARIANT  
<222> (2)..(3)  
<223> Phe, Ile, or Val15 <400> 1  
Phe Xaa Gly Leu Met  
1 520 <210> 2  
<211> 11  
<212> PRT  
<213> Human25 <400> 2  
Arg Pro Lys Pro Gln Gln Phe Phe Gly Leu Met  
1 5 10